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# Theta and Learning: Dorsal and Ventral Hippocampal Theta Oscillation Respond Differently to Learning

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Theta and Learning:  
Dorsal and Ventral Hippocampal Theta Oscillation Respond Differently to Learning

**Greg Newman**

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Greg Newman

University of Connecticut, 2014

## **Abstract**

Rhythmic oscillations within the hippocampus are thought to synchronize various subregions during learning, maximizing efficiency of the neural circuits. In rats, the most prominent oscillation is hippocampal theta. Theta is known to be modulated by an animal's velocity, but it has also been shown to change in response to cognitive demands. To determine if theta is important for learning and memory, EEG recordings were examined for changes in theta related to the decision point of a spatial or non-spatial T-maze task. Additionally, a straight runway served as a control to verify the consistent relationship between velocity and theta during a task with no cognitive demands. Dorsal theta increases during decision points of a spatial task, while ventral theta increases during decision points of a non-spatial task. The relationship between theta and velocity was consistent during the runway task. Once the animal was familiar with the task, theta no longer changed during the decision. The data suggests that theta is responsive to learning, independent of changes in velocity.

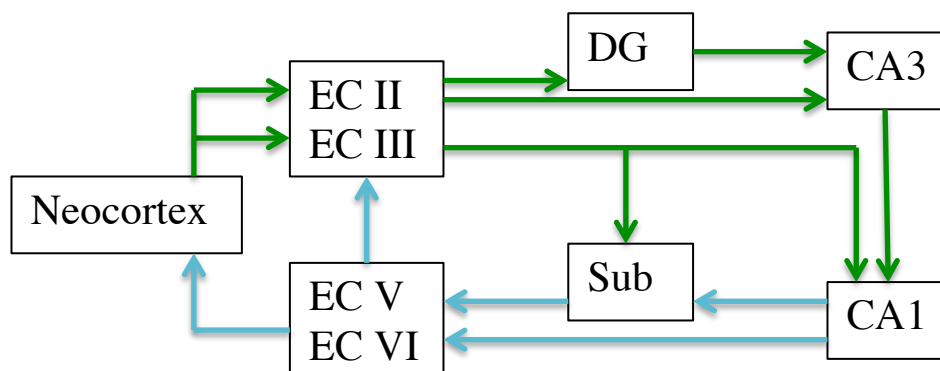


## Introduction

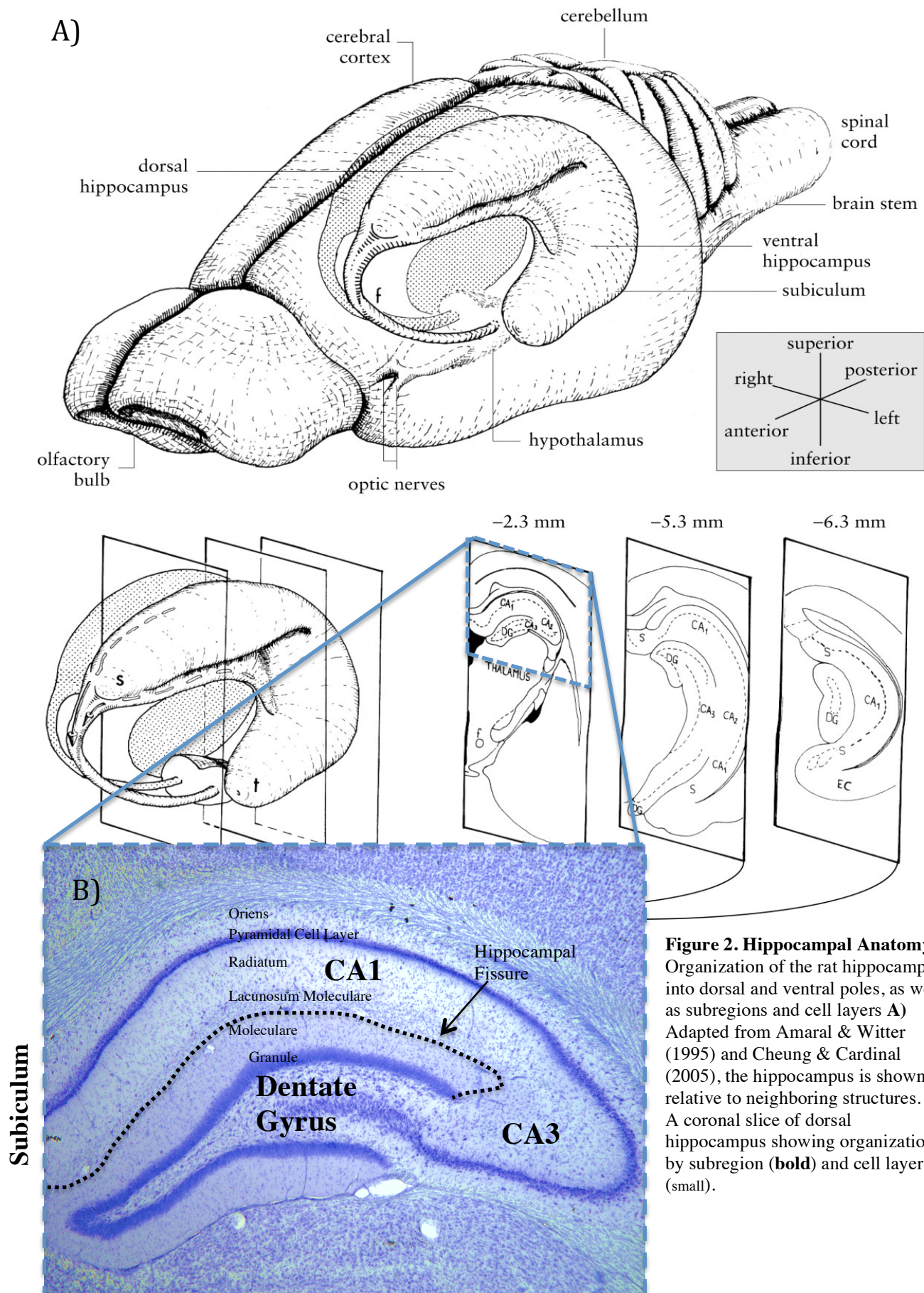
The hippocampus is a key neural substrate for memory and navigation. Part of the limbic system, the human hippocampus is found in the medial temporal lobe, below the cortex. Damage to the human hippocampus can result in the inability to form new episodic memories (Scolville & Milner, 1957). Also, in rodents the hippocampus has been shown to be involved in memory and to act as a cognitive map. For example, an intact hippocampus is required for rodents to learn spatial memory tasks such as the Morris water maze (Morris et al, 1982).

The cytoarchitecture of the hippocampus is complex. It is divided into three constituent parts: dentate gyrus (DG), CA1-CA3, and subiculum, all of which are divided into distinct cellular lamella. Most of the cortical input to the hippocampus comes from the nearby entorhinal cortex (EC), which receives its input from other cortical regions. The EC layer II cells that project to DG and CA3 are known as the perforant path; other inputs from EC layer III innervate CA1 and subiculum (Anderson et al, 1971; Amaral & Witter, 1986). CA1 and subiculum are the main output of the hippocampus, projecting back to EC but synapsing on layers V and VI, which project back to neocortex (**Figure 1**).

The hippocampus also has cytoarchitectural and functional differences between the dorsal and ventral poles (**Figure 2**); most notably larger place fields lower on the septotemporal axis (Moser et al, 1993; Jung et al, 1994; Moser & Moser, 1998). The firing of all these place cells is modulated by grid cell projections from EC that also fire in a spatially dependent manner and may create rhythmicity (Buzsáki, 2002). The difference in place field size suggests that ventral hippocampus holds a representation of the whole environment, while dorsal hippocampus holds representations of smaller zones within that environment (Moser et al, 2008). The action and interaction of these circuits allows both encoding of the environment and integration of the path taken through it.

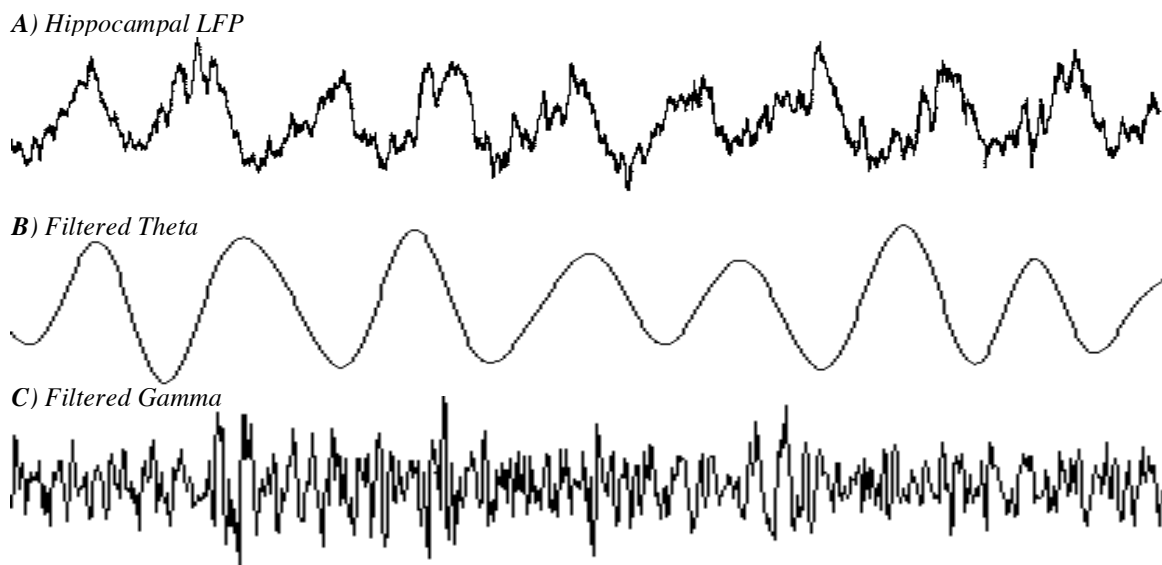


**Figure 1. Hippocampal Connectivity.** A schematic representation of hippocampal circuitry. All synapses shown are glutamatergic. Inputs upstream of CA1 are shown in green, downstream outputs of CA1 are shown in teal. EC, entorhinal cortex; DG, dentate gyrus; Sub, subiculum.



To study the hippocampus, electroencephalographic (EEG) recordings can be used to examine neural activity within certain regions of the brain in the awake, behaving animal. Unlike single unit or multi-unit recordings, which record data from one or many cells, EEG recordings capture local field potentials (LFPs). LFPs reflect gross activity from an area of brain as large as several hundred microns; they can be thought of as summed synaptic activity in the area surrounding the tip of the electrode (Kajikawa & Schroeder, 2011). One prominent oscillation within the hippocampus of both humans and rodent is called theta (Buzsáki, 2002). If the circuits discussed above are thought of as being necessary for forming declarative and spatial memories, then theta can be thought of as the temporal modulator that induces greater efficiency in those circuits.

Theta was first described as a roughly sinusoidal pattern observed when animals reacted or attended to a stimulus (**Figure 3**); theta was also observed following direct electrical stimulation of certain subcortical structures (Green & Arduini, 1954). Theta in freely behaving animals is found between 4 – 12 Hz, with the peak frequency contained within 6 – 8 Hz (Buzsáki, 2002; Hasselmo, 2005). Theta is known to be associated with an animal's velocity; a significant increase in theta is observed when an animal goes from sitting to running (Vanderwolf, 1969; Hinman et al, 2011; Schmidt et al, 2013).



**Figure 3. Example Oscillations.** Oscillations within hippocampus. **A)** An example hippocampal trace, showing the superposition of theta and gamma oscillations. Note that rhythmic theta is clearly visible. **B)** Filtered theta oscillation, occurring between 4 – 12 Hz **C)** Sharp gamma spikes and ripples (25 – 140 Hz) are also found in hippocampus.

Theta characteristics vary within the hippocampus according to cell layer; amplitude is greatest in CA<sub>1</sub> stratum lacunosum moleculare (Buzsáki, 2002). Amplitude decreases as the electrode approaches and passes through the hippocampal fissure before increasing again in the dentate gyrus (Winson, 1974). GABAergic projections from the basal forebrain onto GABAergic interneurons in the hippocampus have been shown to modulate theta (Sava & Markus, 2008). These projections may deteriorate in age, causing the decreased theta power and frequency observed in aged animals (Jacobson et al, 2013).

Theta exhibits different characteristics between dorsal and ventral hippocampus, mirroring the cytoarchitectural and functional differences discussed earlier. Single-unit recordings from ventral hippocampus found that theta power and the number of theta-rhythmic neurons are reduced compared to dorsal hippocampus (Royer et al, 2010). Schmidt et al (2013) showed that while cognitive demands induce a frequency shift in both dorsal and ventral hippocampus, the effect was larger in the dorsal hippocampus. Coherence—a measure of synchronous activity—is known to decrease along the septotemporal axis of the hippocampus (Sabolek et al, 2009). Changes in theta power and coherence in response to behavioral tasks indicate theta is involved in memory from the cellular to the cognitive level.

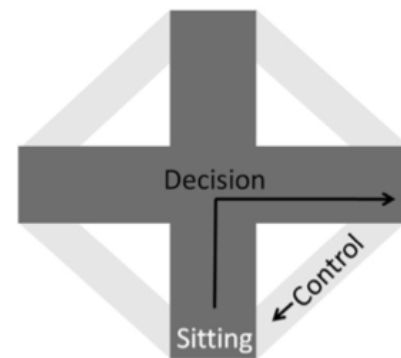
Human theta is purported to assist with mnemonic processes. Magnetoencephalographic (MEG) recordings in humans have shown that recall of information is more successful if theta amplitude is high during learning (Guderian et al, 2009). In a working memory task theta power in the prefrontal cortex of humans has been shown to increase with the amount of information being recalled (Jensen & Tesche, 2002). Kahara et al (1999) recorded intracranial EEG (iEEG) from epileptic patients temporarily implanted with electrodes to localize the source of their seizures. They reported theta increased with complexity of a virtual maze navigation task, and was more prominent during recall than learning. Another iEEG study found that theta increased during all virtual movement, independent of spatial or non-spatial task (Ekstrom et al, 2005).

A function for cerebral rhythmicity has been proposed based on correlations between theta and behavioral task demands or between theta and physiological data. Larson et al (1986) found that a tetanus administered in theta frequency to rat hippocampal slices was most effective at inducing LTP. Winson (1978) showed that the amount of behavioral impairment associated with lesion was correlated with amount of reduction in hippocampal theta. Berry and Thompson (1978) observed a significant predictive effect of hippocampal EEG theta characteristics on the

rate of learning classical conditioning in rabbits. Blockade of hippocampal theta has been shown to impair learning of a Morris water maze task; performance was attenuated using electrical stimulation to restore rhythmicity (McNaughton et al, 2006). Paz et al (2008) suggests that theta functions to impart rhythmicity and synchrony on medial prefrontal neuron populations, allowing transient memory traces from hippocampus to form stable, persistent memories in the cortex.

Recent studies have continued to suggest functions for theta based by showing uncoupling with velocity in response to novelty or during purported decision points (DeCoteau et al, 2007; Montgomery et al, 2009; Jacobson et al, 2013; Schmidt et al, 2013). Jacobson et al (2013) showed that theta increased while velocity decreased when an animal was encoding a novel environment, an effect much smaller in aged animals. DeCoteau et al (2007) showed strong power increases within the theta band up to and during choice arm selection—the purported decision epoch—in a T-maze task. Theta was no longer elevated once the animal reached the goal. Montgomery et al (2009), using a modified T-maze, found that theta power was high while velocity was low in the point leading up to a cued decision; after the decision point theta power was back down and velocity was up.

Schmidt et al (2013) recorded from dorsal and ventral hippocampus while an animal navigated a T-maze using either a spatial or non-spatial strategy and found an uncoupling of dorsal theta power from velocity during the purported decision point of the task (**Figure 4**). Theta power in the dorsal hippocampus during the decision segment of the task was observed to increase compared to a control segment; while theta power in the ventral hippocampus during the decision tended to decrease compared to control. These effects were observed independent of whether or not the animal was using a spatial or non-spatial strategy. Theta coherence between dorsal and ventral hippocampus all appeared to increase during the decision epoch of a spatial task, which was not seen in the non-spatial task. Theta also appeared to change with learning. The difference in dorsal theta power between decision and control became smaller as the animal grew more familiar with the task. While changes in dorsal theta were independent of velocity, changes in ventral theta showed a correlation with the



**Figure 4. Modified T-Maze.** The decision and control epochs of a T-maze choice task used by Schmidt et al (2013). Animals navigated the maze using either a spatial or non-spatial strategy.

speed of the animal. The findings of Schmidt et al (2013) suggest that not only is theta responsive to volitional movement—as has been classically shown—it is also affected by the cognitive demands placed on an animal.

These findings have all led to the prevailing theory that theta serves as a spatiotemporal modulator. The periodic nature of theta oscillations applies a temporal constraint to populations of cells, allowing spike-dependent plasticity (Wittenberg & Wang, 2006). Variations in the power, frequency, and phase of theta allow the hippocampus to encode behavioral information from a time line of seconds to neural information on a time line of milliseconds, a phenomenon known as temporal compression (Skaggs et al, 1996). Taken together, this allows the neural wiring underlying behavior to change in response to feedback being received from the environment.

This study extends upon Schmidt et al (2013) and aims to investigate in a systematic manner how theta is affected during the learning of a spatial or non-spatial task. Recordings were made that examined the same type of behavior—the first ten correct trials—in animals learning a novel task, both before and after they have mastered the task. Learning occurs on the first day of each task (pre-criteria) as the animal makes incorrect and correct trials. The second day (post-criteria) the animal has learned the task and completes it easily. Previous attempts to examine this issue (Howe, 2012) showed that animals were not sufficiently motivated to run the plus maze task over several consecutive days. Animals tended to make multiple errors (pre-criteria), which caused them to slow down and fail to complete the task. Adding a simple runway task between each plus maze training day helped ensure consistent motivation.

#### *Runway*

It was expected that velocity as well as theta power and frequency will increase from the home cage to the runway, as Howe (2012) showed this effect. Velocity and theta power are expected to decrease from the first trials of the day to the last trials of the day. Theta in dorsal hippocampus is expected to have greater power than in ventral hippocampus.

#### *Plus Maze*

Animals are expected to run faster during the decision segment than the control segment. It is expected that dorsal theta power will be higher during the decision segment than during the control segment of the task regardless of spatial or non-spatial strategy. By contrast, ventral theta power is expected to be lower during the decision segment than the control segment, regardless

of task. It is expected that changes in theta on either task will be greater on the pre-criteria day, as Schmidt et al (2013) showed that changes in theta decreased the more familiar an animal was with the task. By contrast, velocity is expected to increase as familiarity with the task increases, as Schmidt et al (2013) showed this effect.

## **Methods**

Methods used were as described in Schmidt et al (2013) with revisions as noted below.

*Subjects.* Five male virgin Fisher 344 rats (Harlan) were used. Rats were received at 3 months old and were housed in a vivarium maintained at ~25°C and kept on a 12 h light/dark cycle (lights on at 08:00). Rats were housed individually in clear Plexiglas cages (46 x 20 x 23 cm) with pine bedding and unrestricted access to water. Rats were maintained at ~85% of their *ad libitum* weight during the experiment and were allowed modest gains to account for growth. Rats were weighed prior to testing each day. All rats weighed between 350 – 400 grams at the time of recording. All procedures were performed in accordance with the University of Connecticut's Institutional Animal Care and Use Committee.

*Apparatus.* A gray, textured plastic runway (120.7 x 10.2 cm) was used for pretraining. Training and recording were on a modified version of the plus maze. The plus maze was constructed of textured gray plastic (112.4 cm long, 10.8 cm wide, 15.9 cm off the table). Four moveable plastic runways were constructed to form a perimeter around the plus maze.

*Runway pretraining.* Rats were trained to run back and forth on a linear runway (**Figure 5A**) for chocolate sprinkle rewards daily for ~3 months. The daily training sessions continued up to 10 min on the runway or until the rat completed 50 trials. After reaching criteria, rats were run twice weekly until needed for surgery. Following surgery rats were given one week to recover and then trained for one week on the runway to ensure surgery did not interfere with their ambulatory ability.

*Surgery.* Rats were anesthetized with isofluorine anesthesia (2-3% isofluorine with 100% O<sub>2</sub>) and placed in a stereotaxic apparatus (ASI Instruments). The scalp was shaved and betadine was applied to the scalp and ophthalmic ointment to the eyes. An incision was made in the scalp and



several small anchor screws were fastened to the skull. For three rats, two electrode arrays (50  $\mu$ m tungsten wires; California Fine Wire) were implanted into the ipsilateral dorsal (A/P -4.0/, L/M  $\pm$ 2.5, D/V 2.5) and ventral (A/P -5.8/, L/M  $\pm$ 5.5, D/V 5.5) hippocampus according to Paxinos and Watson (1986). Two rats were implanted with two electrode arrays (50  $\mu$ m tungsten wires; California Fine Wire) into the ipsilateral dorsal hippocampus (A/P -4.0/, L/M  $\pm$ 2.5, D/V 2.5), two arrays into ipsilateral ventral hippocampus (A/P -5.8/, L/M  $\pm$ 5.5, D/V 5.5) and one array into the pre-limbic area (A/P +3.2/, L/M  $\pm$ 0.5, D/V 2.5). Electrodes comprised four wires spaced evenly along the medial–lateral axis, cut at a slight angle, and arranged with fused silica tubing (Polymicro Tubing, Phoenix, AZ). After implantation, electrodes were secured with dental acrylic. Hippocampal electrodes were targeted to CA1. The same electrodes were used for all analyses. Two ground screws were placed over the cerebellum to use as reference and ground. Rats received the analgesic Metacam 1 mg/kg (oral) after surgery and for the following two days. After surgery, the rats were given penicillin G procaine (i.m.) to prevent infection. The animals were placed in a clean cage with a heating pad until ambulatory, after which they were single housed in clean cages with bedding. The animals were allowed one week to recover before finishing pretraining and beginning recording.

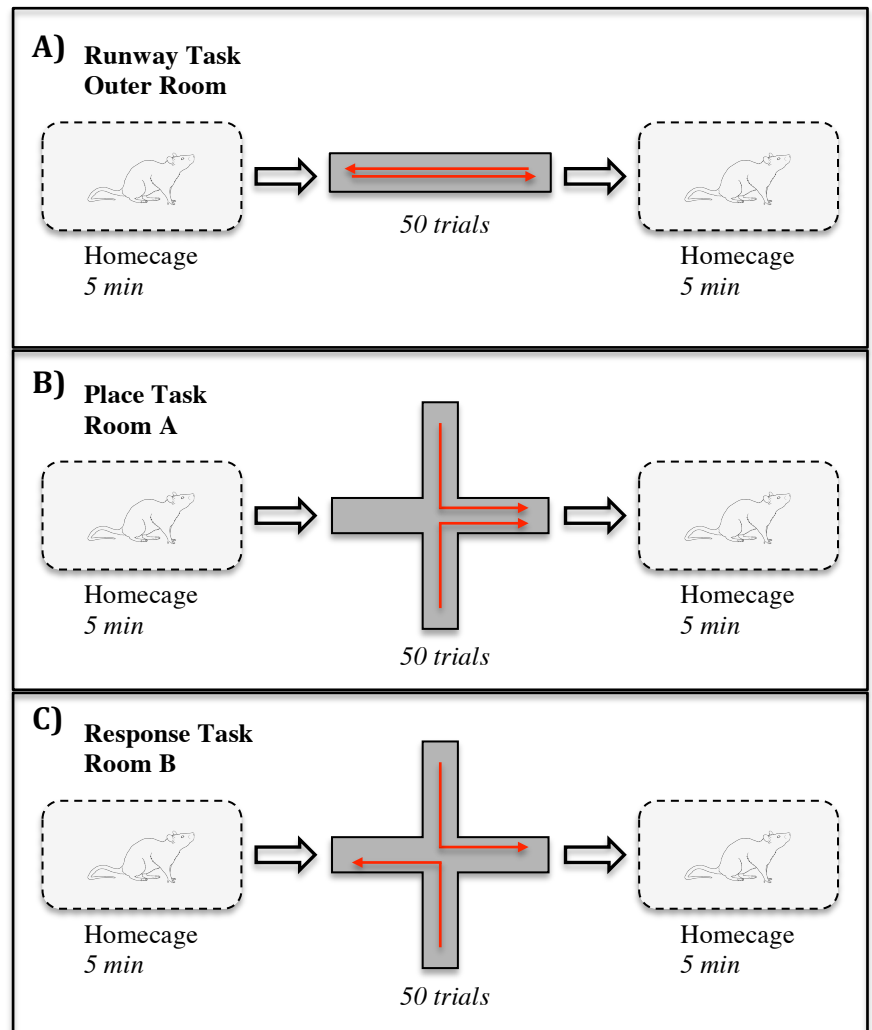
*Plus maze procedure.* Rats were recorded on a plus maze in a dimly lit room. Animals were placed on the start arm behind a barrier. The trial started when the barrier was lifted and ended as soon as the rat reached the feeder of a choice arm. Once a trial ended, the rat was blocked from re-entering the maze and allowed to run the perimeter runways to the next start arm (**Figure 4**). The perimeter allowed the animal to return to the start arm after each trial without traversing back through the maze. This was done to minimize stress by reduced experimenter handling of the animal. During the place (spatial reference task) task the animal was rewarded for going to the same place (east arm) regardless of start arm (**Figure 5B**). During the response (fixed task) the animal was rewarded for making a left turn regardless of start arm (**Figure 5C**).

*Experimental procedure.* Each recording session consisted of 50 trials or until the animal would no longer behave, whichever came first. Rats were recorded for five minutes in their homecage before and after the task to establish a baseline (**Figure 5**). Reaching criteria was considered nine out of ten trials correct, after which the rat would do one more day of the same task. Rats were first recorded on the runway task, then either place or response alternating with runway until

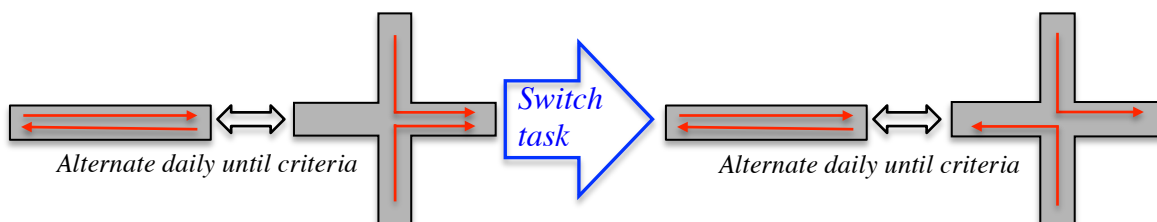


criteria was reached, after which they completed the other task alternating with runway until criteria was reached (**Figure 6**).

**Recordings.** For three rats, wide-band electrical activity was recorded (1–2000 Hz, 3787 samples/s) using Neuralynx Data Acquisition System. For two rats, wide-band electrical activity was recorded (1–9000 Hz, 30,296 samples/s) using Neuralynx Data Acquisition System. All files were downsampled to 473 samples/s using Neuralynx Rate Reducer. Light-emitting diodes attached to the headstage were tracked with an overhead camera (33 samples/s) and monitored with the Neuralynx Video Tracker. Data were selected and analyzed offline.



**Figure 5. Behavioral Procedure.** All recordings began and ended with 5 minutes in the homecage and were conducted in unique rooms. Criterion was considered to be nine out of ten trials correct. Correct arms were baited with chocolate sprinkles. **A)** Animals completed 50 alternations on a straight runway. **B)** Animals completed 50 trials of a spatial reference ‘place’ task. **C)** Animals completed 50 trials of a fixed ‘response’ task.



**Figure 6. Experimental Procedure.** Animals were recorded every other day on a plus maze task they reached criteria. On days not recording from the plus maze, animals were recorded on the runway alternation task. Once criteria was reached on the first task animals switched to the second task. Animals underwent pretraining on the runway task.

*Data Selection.* Pre-criteria was defined as the first ten correct trials on the day the animal completed at least twenty trials—with nine out of ten correct (criteria) somewhere within—for the first time. Post-criteria was defined as the first ten correct trials in a row from the session following the one in which the animal reached criteria. In this manner, pre-criteria data was composed of trials that saw the animal learning the task; incorrect trials were frequently interleaved. By contrast, post-criteria was composed of ten successive, correct trials—most often the first ten of the day—that showed the animal was comfortable with the task, and no learning was occurring. This selection allowed observation of the effects of spatial and non-spatial cognitive engagement on theta.

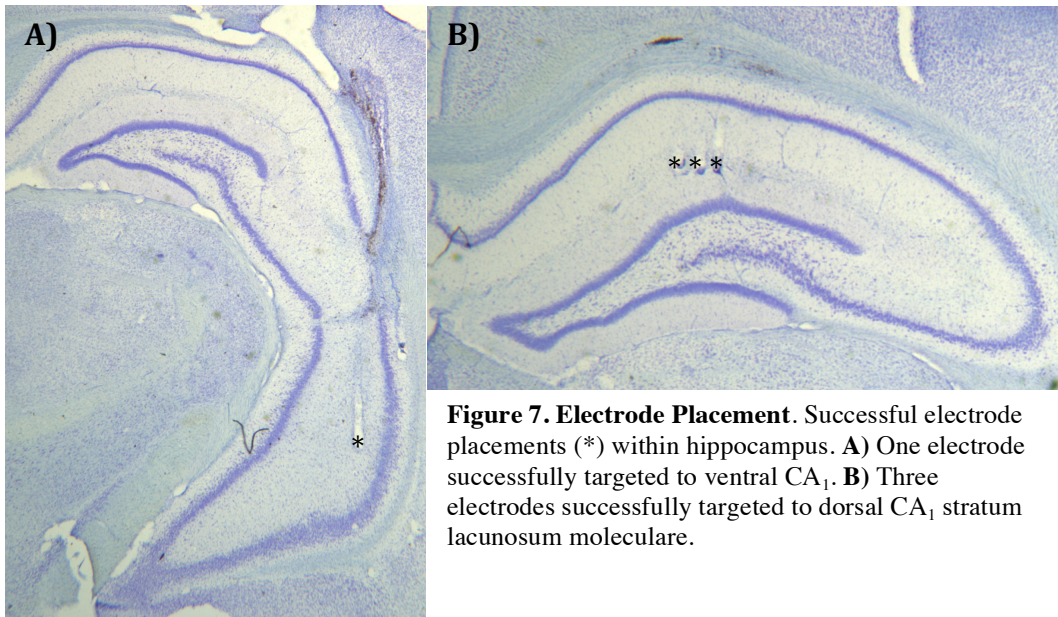
*Analysis.* All data were initially inspected visually (Neuraview, Neuralynx) to remove segments of bad signal (e.g., due to a loose connection, bumping head). All signal analysis was conducted using custom-written programs in MATLAB (MathWorks). Data was flagged during recordings to select specific behavioral epochs, and flag locations were confirmed using Neuralynx Video Tracker File Playback and Neuraview. Segments selected were running to the start arm (control epoch) and running to the goal arm (decision epoch), each truncated to 1.5 seconds for analysis. Running speed for each was calculated as the positional difference between successive tracking samples and then low-pass filtered (cutoff = 0.25 Hz) to minimize the contribution of head movements and movement artifacts to the overall speed. Power spectral density estimates were obtained in MATLAB using Welch's averaged modified periodogram method (Welch, 1967). Each session was then blocked and power and frequency estimates were obtained for each segment of every trial. Power estimates were obtained for the theta band (6 –10 Hz) and represented as decibels (dB) relative to 1  $\mu$ V. Statistical analyses performed in SPSS 21 (IBM).

*Histology.* Rats were perfused and examined for verification of electrode locations. Rats were killed in a carbon dioxide chamber and transcardially perfused with 100 ml of saline followed by 400 ml of 4% fresh paraformaldehyde. Brains were removed and stored in fixative overnight. The brains were placed in a 30% sucrose solution for 3 days. The brains were frozen and coronally sliced at 60  $\mu$ m on cryostat and stained with thionin.

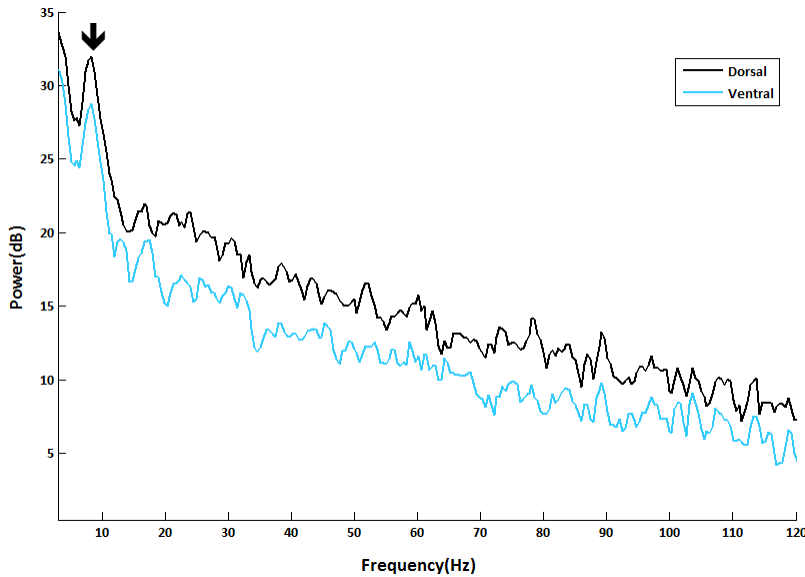
## Results

A total of five rats were implanted with electrodes and trained on the two tasks, The velocity data (**Table 1**) is based on four of these animals (719, 722, 724, 726). Of these animals, two rats had dorsal electrodes successfully targeted to CA<sub>1</sub> stratum lacunosum moleculare or stratum radiatum (**Figure 7**) and one was used for LFP analysis (719). Additional sites were recorded from other cell layers in CA<sub>1</sub>, DG, and CA<sub>3</sub>. Ventral electrodes were successfully targeted to CA<sub>1</sub> in three animals, although some sites were recorded from CA<sub>3</sub> in other animals. Theta oscillations were present in sites in dorsal and ventral CA<sub>1</sub> as evidenced by the clear power spectral density peak (**Figure 8**) around 9 Hz. Dorsal theta was typically greater in power than ventral theta, as can be seen in the figure.

To achieve consistent data between animals all electrodes need to be localized to a common region. For this study, four animals were removed from power analyses due to poor electrode placement. Electrodes were most often found to have penetrated too deeply, past CA<sub>1</sub> and into dentate gyrus. Some electrodes were found too lateral, in CA<sub>3</sub>. Electrodes were also excluded if they terminated in layers with low amplitude theta such as oriens and pyramidal.



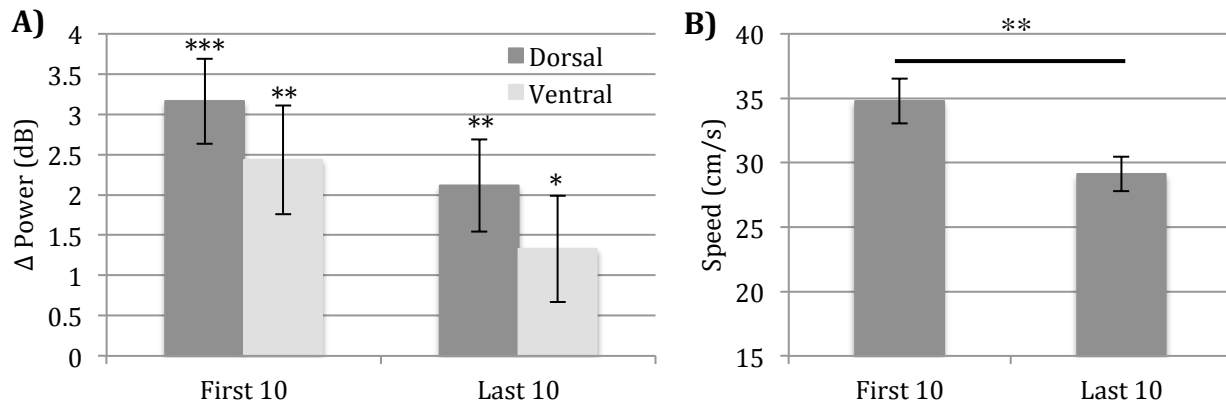
**Figure 7. Electrode Placement.** Successful electrode placements (\*) within hippocampus. **A)** One electrode successfully targeted to ventral CA<sub>1</sub>. **B)** Three electrodes successfully targeted to dorsal CA<sub>1</sub> stratum lacunosum moleculare.



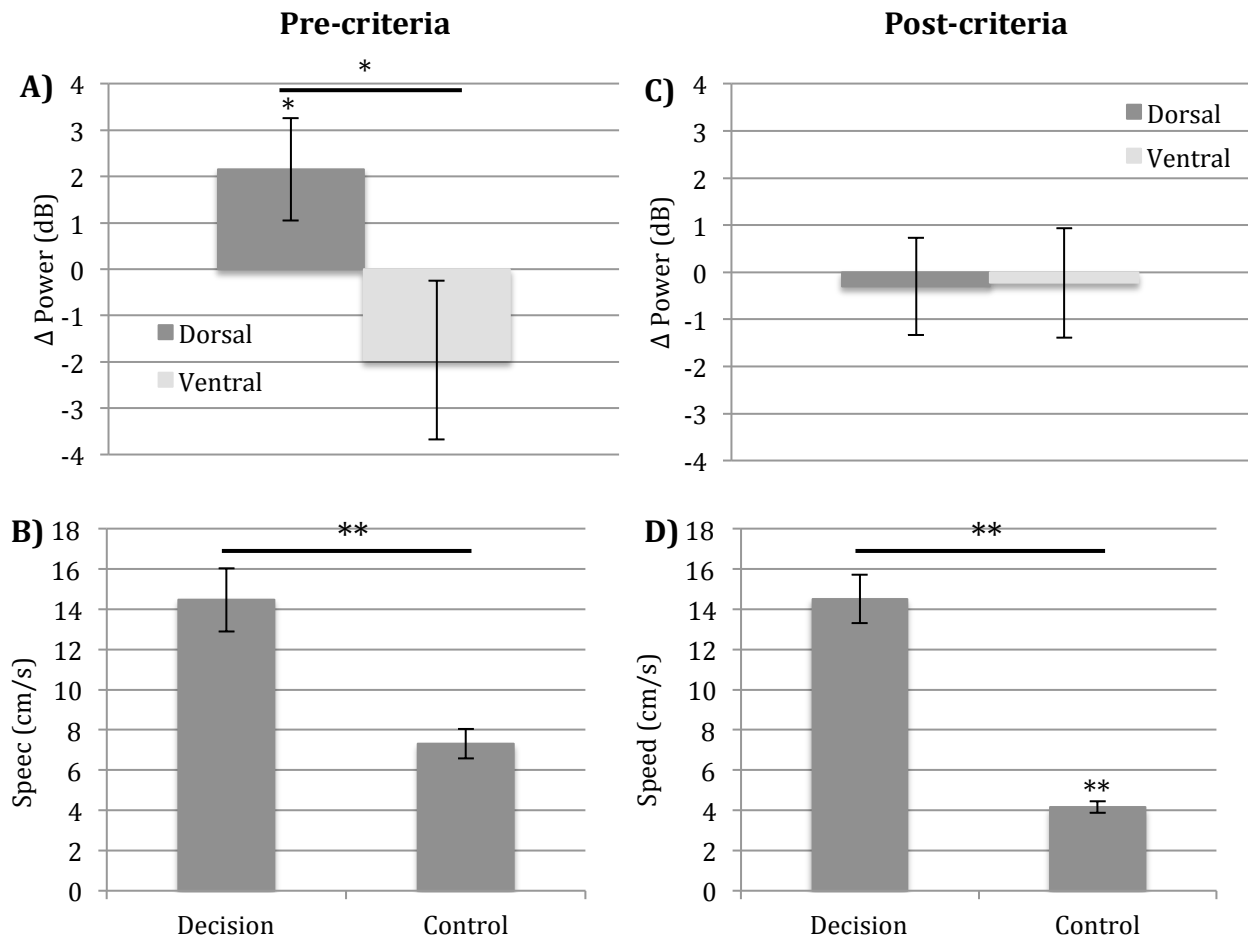
**Figure 8. Power Spectral Density.** Power spectral density example plot is shown. The peak (↓) at ~ 9 Hz indicates clear theta. Dorsal theta has slightly greater power than ventral theta.

Repeated measures ANOVA revealed a significant overall effect of trial number of velocity ( $F(1,9) = 30.464$ ;  $p < 0.001$ ). The last ten trials were significantly slower (planned comparisons,  $p < 0.01$ ) than the first ten trials. A t test revealed that dorsal theta was elevated compared to homecage during the first ten ( $t_{(9)} = 5.985$ ,  $p < 0.001$ ) and last ten ( $t_{(9)} =$

$3.699$ ,  $p < 0.005$ ) trials. Theta in the ventral hippocampus was also elevated compared to homecage during the first ten ( $t_{(9)} = 3.609$ ,  $p < 0.005$ ) and last ten ( $t_{(9)} = 2.015$ ,  $p < 0.05$ ) trials. A paired samples t test revealed no difference between the elevation in dorsal theta power during the first ten ( $M = 3.16$ ,  $SD = 1.58$ ) and last ten ( $M = 2.11$ ,  $SD = 1.71$ ) trials ( $t_{(10)} = 1.44$ ,  $p = 0.181$ ). There was also no difference between the elevation in ventral theta power during the first ten ( $M = 2.43$ ,  $SD = 2.13$ ) and last ten ( $M = 1.33$ ,  $SD = 2.09$ ) trials ( $t_{(10)} = 1.06$ ,  $p = 0.317$ ). The elevation in dorsal theta was not different from the elevation in ventral theta during the first ten ( $t_{(9)} = 1.063$ ,  $p = 0.316$ ) or last ten ( $t_{(9)} = 0.806$ ,  $p = 0.441$ ) trials.

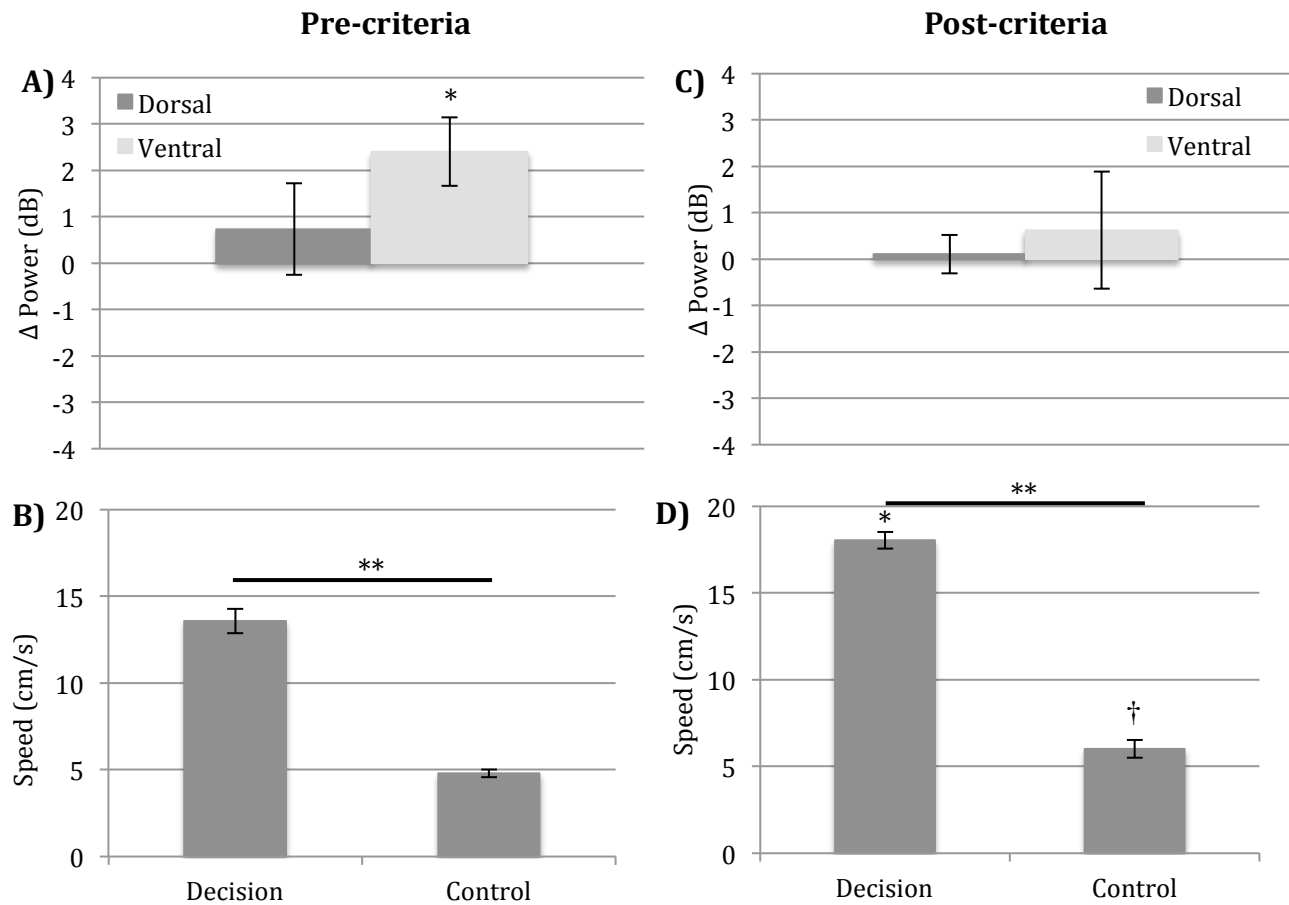


**Figure 9. Runway Task.** The relationship between theta and velocity is consistent for a task with no cognitive demands. Data shown is from one animal. **A)** Mean ( $\pm$ SEM) change in theta power from homecage to the first ten and last ten trials of the runway. **B)** Mean ( $\pm$ SEM) velocity during the first ten and last ten trials. Typical velocity for homecage was 2 cm/s. \*\*\* $p = 0.001$ , \*\* $p = 0.01$ , \* $p = 0.05$ .



**Figure 10. Spatial Task.** The relationship between theta and velocity is uncoupled during a task with cognitive demands. Data shown is from one animal. **A)** Mean ( $\pm$ SEM) change in theta power during decision compared to control on the day the animal reached criteria. **B)** Mean ( $\pm$ SEM) speed of animal during decision and control epochs. **C)** Mean ( $\pm$ SEM) change in theta power during decision compared to control the day after the animal reached criteria. **D)** Mean ( $\pm$ SEM) speed of animal during decision and control epochs. \*\* $p=0.01$ ; \* $p=0.05$ .

Repeated measures ANOVA revealed a significant effect of spatial task epoch on velocity on the pre-criteria day ( $F(1,9) = 11.311$ ,  $p < 0.01$ ) as well as the post-criteria day ( $F(1,9) = 79.338$ ,  $p < 0.001$ ). The animal ran significantly slower in the control segment of the task (planned comparisons,  $p < 0.01$ ) than the decision segment on both days. Theta power within the dorsal hippocampus was elevated compared to control on the pre-criteria day ( $t_{(9)} = 1.952$ ,  $p < 0.05$ ) but not the post-criteria day ( $t_{(9)} = -0.292$ ,  $p > 0.1$ ). Ventral theta power did not change from the decision to control epoch on the pre-criteria ( $t_{(8)} = -1.09$ ,  $p > 0.1$ ) or post criteria day ( $t_{(9)} = -0.194$ ,  $p > 0.1$ ). The change in dorsal theta power was greater than the change in ventral power on the pre-criteria day ( $t_{(8)} = 2.40$ ,  $p < 0.05$ ) but not the post-criteria day ( $t_{(10)} = -0.052$ ,  $p > 0.1$ ). The animal ran slower on the post-criteria day during the control ( $t_{(9)} = 4.510$ ,  $p < 0.01$ ) but not decision ( $t_{(9)} = -0.040$ ,  $p > 0.1$ ) segment.



**Figure 11. Non-Spatial Task.** The relationship between theta and velocity is uncoupled during a task with cognitive demands. Data shown is from one animal. **A)** Mean ( $\pm$ SEM) change in theta power during decision compared to control on the day the animal reached criteria. **B)** Mean ( $\pm$ SEM) speed of animal during decision and control epochs. **C)** Mean ( $\pm$ SEM) change in theta power during decision compared to control the day after the animal reached criteria. **D)** Mean ( $\pm$ SEM) speed of animal during decision and control epochs. \*\*p=0.01; \*p=0.05; †p=0.1.

Repeated measures ANOVA revealed a significant effect of non-spatial task epoch on velocity on the pre-criteria day ( $F(1,9) = 131.577$ ,  $p < 0.001$ ) as well as the post-criteria day ( $F(1,9) = 469.458$ ,  $p < 0.001$ ). The animal ran significantly slower in the control segment of the task (planned comparisons,  $p < 0.01$ ) than the decision segment on both days. Theta power within the dorsal hippocampus did not vary during decision and control segments on the pre-criteria day ( $t_{(9)} = 0.740$ ,  $p > 0.1$ ) or the post-criteria day ( $t_{(9)} = 0.200$ ,  $p > 0.1$ ). Ventral theta power was elevated from the decision to control epoch on the pre-criteria day ( $t_{(9)} = 3.251$ ,  $p < 0.05$ ) but not the post criteria day ( $t_{(9)} = 0.494$ ,  $p > 0.1$ ). The increase in ventral theta power was not greater than the change in dorsal power on the pre-criteria day ( $t_{(9)} = 1.593$ ,  $p > 0.1$ ) and the two were the same on the post-criteria day ( $t_{(9)} = 0.429$ ,  $p > 0.1$ ). On the post-criteria day the animal ran faster during the decision ( $t_{(9)} = -5.91$ ,  $p < 0.01$ ) and tended to run faster during the control ( $t_{(9)} = -2.076$ ,  $p < 0.1$ ) segment than the pre-criteria day.

Task/Animal	719 (Place)		722 (Place)		724 (Response)		726 (Place)	
Place	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Decision	14.4 ± 4.9	14.5 ± 3.8	11.8 ± 2.6	15.6 ± 3.5	11.9 ± 2.7	12.6 ± 3.8	12.8 ± 4.1	15.7 ± 4.1
Control	7.32 ± 2.3	4.15 ± 0.92	5.08 ± 1.2	5.55 ± 1.0	5.32 ± 1.1	5.58 ± 1.4	5.53 ± 2.2	5.43 ± 1.0
Response								
Decision	13.5 ± 2.2	18.0 ± 1.5	17.2 ± 4.9	19.9 ± 3.6	14.6 ± 3.6	13.8 ± 3.2	11.3 ± 3.3	20.1 ± 6.4
Control	4.78 ± 0.72	6.02 ± 1.6	5.72 ± 1.0	5.06 ± 0.72	5.45 ± 1.1	5.96 ± 1.4	5.32 ± 1.4	7.23 ± 1.2

**Table 1.** Mean ( $\pm$ STD) velocity for each animal for each epoch on both pre- and post-criteria days. Animals typically ran the same speed or faster on post-criteria days, with some individual variability. The task the animal learned first is shown next to their animal number.

## Discussion

This paradigm proved to be a valuable method for studying how hippocampal theta changes in response to learning, and the runway alternation task served as a valuable control that showed the relationship between velocity and theta is consistent during a task with no cognitive demands (**Figure 9**). Theta power increased from the homecage to the runway, as expected (Howe, 2012; Jacobson, 2013). This increase in theta is due to the increased velocity of the animal, from sitting in the homecage to running on the runway. Velocity was observed to decrease over the course of the trials, and a similar reduction was seen in theta power. It is important to note that both dorsal and ventral theta power increased during the runway alternation task. While dorsal theta appears to have increased to a greater degree than ventral theta (**Figure 9A**), the difference was not significant. These results align with previous research (Vanderwolf, 1969; Hinman et al, 2011; Schmidt et al, 2013) that reported a correlation between theta power and velocity.

The results of both plus-maze tasks suggest that theta responds very differently when cognitive demands are applied to the animal. On the spatial task (**Figure 10**), the animal ran faster during the decision segment than the control segment on both days (**Figure 10 B,D**). On the post-criteria day, the animal ran slightly slower during the control segment than on the pre-criteria day. This difference was small and atypical, as **Table 1** shows. All other animals either ran faster or the same speed on post-criteria days. On the pre-criteria day, dorsal theta power was elevated during the decision segment of the task (**Figure 10A**), both compared to the control segment and compared to the change in ventral theta power during the same epoch. While ventral theta appeared to decrease, the difference was not significant. These findings are mostly in keeping with Schmidt et al (2013), which showed that dorsal theta increases and ventral theta

decreases during a novel spatial task. On the post-criteria day, there was no difference between dorsal or ventral theta power during the decision and control segments of the task (**Figure 10C**). This is an important validation of the effects of learning on theta power. It was expected that on the pre-criteria day dorsal theta would be elevated during the decision segment of the task, as shown by Montgomery et al (2009) and Schmidt et al (2013). However, on the post-criteria day theta is no longer elevated during the decision, because the animal is familiar with the task and is no longer learning it.

The findings of the non-spatial task were different than expected (**Figure 11**). As in the spatial task, the animal ran slower during the control segment than the decision segment on both days (**Figure 11 B,D**). Unlike the spatial task, the animal ran faster during on the post-criteria day during the decision segment, and there was a trend for greater speed during the control segment as well. This change in velocity mirrors the learning of the task. On the post-criteria day, when the animal is familiar with the task it completes it faster and easier. Theta in the non-spatial task responded in an almost-opposite manner to the spatial task. On the pre-criteria day, ventral theta power was elevated during the decision segment, while dorsal theta did not distinguish between the decision and control segments (**Figure 11A**). These findings are in contrast to Schmidt et al (2013) who showed a reduction in ventral theta accompanied by an increase in dorsal theta during the decision segment of a response task. Further research should be completed to explain the discrepancy between these findings. Results from the post-criteria day were more aligned with expectations (**Figure 11C**). Neither dorsal nor ventral theta distinguished between the decision and control segments. This is in keeping with the findings of the spatial task, which showed that once an animal has learned a task, theta is no longer elevated during the decision point of that task.

It is important to note that neither pre-criteria day was the animal's first day on that plus maze, as Jacobson et al (2013) showed an effect of novel environment on theta power that could have confounded the changes in theta that were associated with learning.

A challenge in any electrophysiology study is achieving consistent electrode placement between animals. Theta is notoriously different between different cell layers of hippocampus. Theta within stratum oriens and the pyramidal cell layer has such low amplitude as to be almost undetectable; amplitude increases with electrode depth from stratum oriens to stratum lacunosum moleculare and undergoes a 180° phase shift, such that peaks in oriens are in



phase with troughs in lacunosum moleculare (Buzsáki, 2002). Amplitude decreases again through the hippocampal fissure (Winson, 1974). Although theta can be recorded from the dentate gyrus, the signal contains a higher percentage of gamma, and therefore less theta (Buzsáki, 2002; Sabolek et al, 2009). These variations in theta amplitude and phase make it difficult to achieve consistent data.

Variations have also been reported in the character of theta within cell layers of dorsal hippocampus. Montgomery et al (2009) reported striated patterns of current sources and sinks between and within cell layers, like a checkerboard pattern overlaid on a coronal slice of dorsal hippocampus. While spike timing was well synchronized between sources in the same cell layer, sources in different cell layers were phase shifted. This has led to the suggestion that multiple, relatively independent sources of theta generation exist within hippocampus and that variations in coherence between any combination of these dipoles subtly distinguishes between and modulates the numerous behaviors theta is associated with (Buzsáki, 2002; Montgomery et al, 2009). Unfortunately, these alternating sources and sinks lead to power changes between electrode locations that introduce variability into the data, making it more difficult to distinguish clean differences. The inclusion of more electrodes would have lent greater statistical power to the results, allowing firmer conclusions to be drawn regarding the effect of learning on theta.

Another challenge in this study was the behavior of the animals. Being placed on an open arm elevated plus maze for the first time magnifies the differences in the individual anxiety levels of the animals and can create behavioral issues (Pellow et al, 1985). Calm animals may complete 50 trials on the first day, while anxious animals may refuse to run on the plus maze for several days before starting to complete trials. To correct for this, specific data were selected that were consistent between animals (see *Data Selection*, **Methods**).

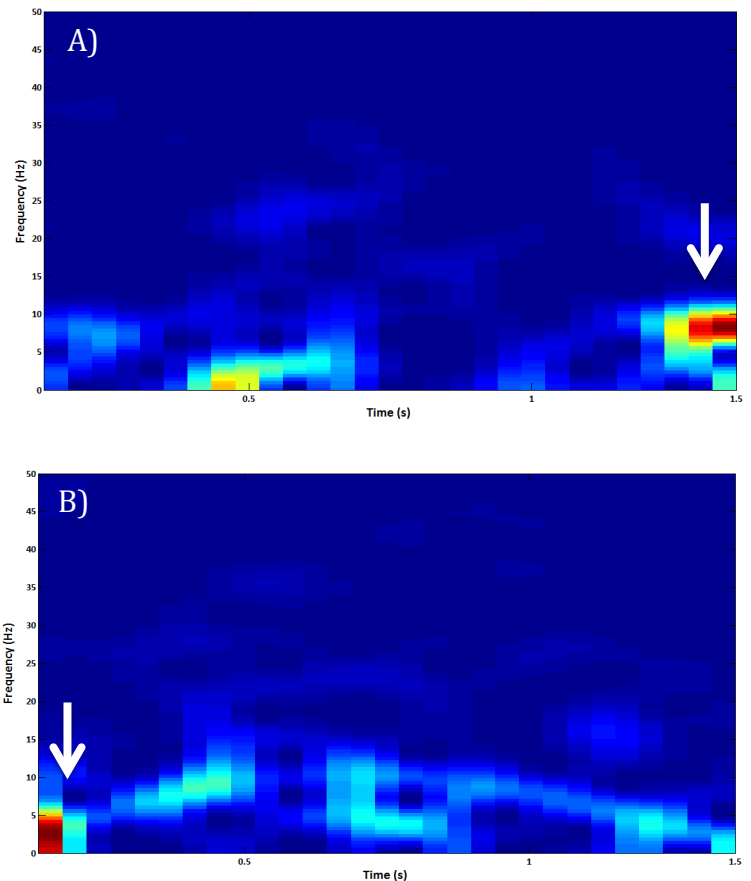
A methodological challenge posed to this study was the selection of decision and control epochs. The decision epoch was truncated to 1.5 seconds from the moment the animal began running toward the intersection; the control epoch was truncated to 1.5 seconds from the moment the animal started running to the next starting arm. Segments were shortened in this manner to control for varying trial length. As a result, long trials in which the animal engaged in a variety of exploratory behaviors (sniffing, venturing out and back) may have been shortened to a greater extent than would accurately reflect the decision making period. Analysis of certain trials revealed the decision segment was sometimes too short to contain the entire change in theta

associated with the cognitive demand (**Figure 12A**). Analyses of control epochs indicated that theta was sometimes overly elevated early in the segment as the animal attended to the newly available pathway (**Figure 12B**). These phenomena could be avoided and cleaner data could be obtained by lengthening the decision segment and insuring the control segment starts slightly after the diagonal pathway is first made available to the animal. Future research or further analysis of this data should be directed toward determining the most accurate segment length to be used for decision and control analysis.

Future research should be directed toward repeating this paradigm. Results clearly showed an effect of learning on theta independent of an animal's velocity. However, because there were not enough electrodes, no absolute conclusions can be drawn. As discussed previously, further analysis of this data should investigate the best length for the decision and control segments. Rather than demarcating those epochs based on time, selecting based on position of the animal may yield the best results.

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**Figure 12. Spectrogram.** Spectral data between 0 and 50 Hz over 1.5 seconds are shown. **A)** An example decision segment showing that 1.5 seconds truncated the trial prematurely, before the elevation in theta associated with the cognitive demands (↓) was fully observed. **B)** An example control segment showing that theta was briefly elevated (↓) after the return pathway became available to the animal.

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